# MAGNESIUM UTILIZATION IN WETHERS FED DIETS WITH VARYING RATIOS OF NITROGEN TO READILY FERMENTABLE CARBOHYDRATE

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## SUMMARY

Two experiments were conducted to study magnesium utilization in wether lambs fed diets with varying ratios of nitrogen to readily fermentable carbohydrate (N:RFC). Semipurified diets fed to the lambs contained about 4.1% nitrogen (25.6% crude protein), .08% magnesium, .4% calcium, 2.4% potassium and varied amounts of readily fermentable carbohydrate provided mainly as either starch (experiment 1) or sucrose (experiment 2). Dietary ratios of N:RFC were .2, .4, .7 and .9 in experiment 1 (five lambs/treatment), and .2 and 1.6 in experiment 2 (nine lambs/treatment).

In experiment 1, ruminal fluid ammonia-nitrogen levels increased as starch intake decreased, but differences between treatments were not significant ($P>.05$). Apparent absorption, retention and plasma concentration of magnesium were not affected ($P>.05$) by decreased starch intake. In experiment 2, ruminal ammonia levels increased ($P<.05$) as sucrose intake decreased. Urinary excretion ($P<.05$) and apparent absorption ($P<.1$) of magnesium were higher in lambs fed supplementary sucrose than in those fed the diet without added sucrose; plasma magnesium levels were similar in both groups. Results of experiment 2 support the hypothesis that supplemental energy may enhance the availability of dietary magnesium to ruminants.

(Key Words: Magnesium Utilization, Dietary Nitrogen, Readily Fermentable Carbohydrate, Ruminal Ammonia, Plasma Minerals, Sheep.)

## INTRODUCTION

The etiology of hypomagnesemic tetany has been associated with several chemical factors in forage (Molloy, 1971). Applying nitrogen fertilizer generally increases the nitrogen content of forages, and increased nitrogen intake may affect magnesium metabolism in ruminants (Mayland *et al.*, 1975). Decreased utilization of dietary magnesium (Smyth *et al.*, 1958; Stillings *et al.*, 1964) and increased incidence of hypomagnesemia (Kemp, 1960) have been observed in ruminants fed forages fertilized with high levels of nitrogen. However, as indicated by Fontenot *et al.* (1973), these results may be due to some chemical alteration in the plant other than an increase in nitrogen content.

A similar hypothesis is that an imbalance between crude protein and readily available carbohydrates in plants predisposes animals to tetany (Metson *et al.*, 1966). Some support for this hypothesis is apparent from the work of Wilson *et al.* (1969) who found that feeding supplemental starch to dairy cows lessened the depression in plasma magnesium levels observed when the cows grazed "tetany prone" pastures. Moreover, Mayland *et al.* (1974) reported that hypomagnesemic tetany in beef cattle grazing crested wheatgrass (*Agropyron desertorum*) coincided with periods when the ratio of nitrogen to water soluble carbohydrates in the forage increased rapidly and was maximal for the year.

The study reported here was conducted to assess the effect of different dietary levels of readily fermentable carbohydrate on magnesium metabolism in wether lambs fed relatively high nitrogen diets. Effects of the diets on calcium and potassium utilization were determined also.

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## EXPERIMENTAL PROCEDURE

Crossbred, 9-month-old, wether lambs with an average body weight of about 39 kg were used in two metabolism trials. The lambs were fed low-magnesium, semipurified diets provided as wet-mash formed by adding about 150 ml of water to each 100 g of feed; additional water was available *ad libitum*. All data relative to feed intake and dietary composition are on a dry matter basis.

Table 1 shows the ingredient and chemical composition of the experimental diets. All diets contained about 4.1% nitrogen (25.6% crude protein), provided mainly as soybean protein and urea. Dietary levels of readily fermentable carbohydrate were adjusted with either starch (experiment 1) or sucrose (experiment 2) to vary the ratio of total nitrogen to readily fermentable carbohydrate (N:RFC). Dietary levels of nitrogen, potassium, calcium, organic acids and fatty acids were about equal among rations, and were adjusted to approximate the contents reported for "tetany prone" forage (Mayland *et al.*, 1974).

The lambs were confined separately in metabolism crates to facilitate daily collections of urine and feces. During collection periods, total urine and feces, and samples of blood and diets were obtained and handled as described by House and Mayland (1976). Ruminal fluid was obtained through a stomach tube.

Magnesium, calcium and potassium in feed, feces, urine and plasma were determined by atomic absorption spectrophotometry in the presence of lanthanum chloride (Anonymous, 1973). Feed and fecal samples were wet-ashed in a nitric:perchloric acid mixture (3:1, v/v) prior to dilution and analysis. Plasma and urine were diluted appropriately and aspirated directly into the spectrophotometer. Ruminal fluid was filtered, centrifuged and ammonia-nitrogen content determined as described by Bremner (1965). Total nitrogen content of feeds was determined by semimicro-Kjeldahl procedures. Water soluble sugars in each diet were deter-

TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF SEMIPURIFIED DIETS THAT CONTAINED ADDED STARCH (EXPERIMENT 1) OR SUCROSE (EXPERIMENT 2)

| | Diet | | | | | |
|---|---|---|---|---|---|---|
| | Experiment 1 | | | | Experiment 2 | |
| Ingredient | A | B | C | D | E | F |
| | % | | | | | |
| Corn starch (4-02-889)[a] | 18.5 | 7.4 | 3.8 | 1.9 | ... | ... |
| Sugarcane, sugar (4-04-701)[a] | ... | ... | ... | ... | 18.5 | ... |
| Cellulose[b] | 10.4 | 21.5 | 25.1 | 27.0 | 10.4 | 28.9 |
| Mineral mix[c] | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 |
| Premix[d] | 65.9 | 65.9 | 65.9 | 65.9 | 65.9 | 65.9 |
| Vitamins[e] | + | + | + | + | + | + |
| Analysis: | | | | | | |
| Magnesium, mg/kg | 753 | 763 | 758 | 763 | 810 | 810 |
| Calcium, g/kg | 4.12 | 4.19 | 4.16 | 4.16 | 4.18 | 4.16 |
| Potassium, g/kg | 24.24 | 24.24 | 24.11 | 24.11 | 24.37 | 24.65 |
| Nitrogen, % | 4.17 | 4.12 | 4.18 | 4.11 | 4.14 | 4.15 |
| N:RFC[f] | .2 | .4 | .7 | .9 | .2 | 1.6 |

[a]International Reference Number.

[b]Alphacel, Nutritional Biochemicals Corp., Cleveland, OH.

[c]Composition: $CaCO_3$, 5.3 g; $NaHCO_3$, 2.6 g; KCl, 8.6 g; $KHCO_3$, 11.5 g; $K_2HPO_4$, 23.0 g; $FeSO_4 \cdot 7H_2O$, .6 g; $ZnCO_3$, 115 mg; $MnSO_4 \cdot H_2O$, 160 mg; $CuSO_4 \cdot 5H_2O$, 36 mg; KI, 20 mg; $CoCl_2 \cdot 6H_2O$, 1 milligram.

[d]Expressed as percent of total diet, all rations contained: timothy hay (IRN 1-04-889), 40%; sugarcane molasses (IRN 4-04-696), 4%; corn oil (IRN 4-07-882), 4%; urea (IRN 5-05-070), 4%; citric acid (IRN 8-01-233), .9%; and soybean protein (Assay Protein C-1, Skidmore Enterprises, Cincinnati, OH), 13%.

[e]Added per kilogram diet: retinyl palmitate, 1,350 IU; cholecalciferol, 350 ICU; DL-α-tocopheryl acetate, 50 IU.

[f]Dietary ratio of nitrogen to readily fermentable carbohydrates.

mined by the method of Metson *et al.* (1966). Dietary readily fermentable carbohydrate was calculated as the amount of water soluble reducing sugars plus the amount of starch added to the ration. The experimental data were evaluated statistically by either an analysis of variance or a two-sided *t*-test (Steel and Torrie, 1960).

*Experiment 1.* Twenty lambs, stratified by weight to four groups of five each, were used in a feeding trial to determine the effect of decreased starch intake (increased dietary ratio of N:RFC) on magnesium utilization. The trial consisted of a 19-day preliminary period followed by a 7-day experimental period. All lambs were fed the same ration (diet A, table 1) throughout the preliminary period. Initially, the sheep were fed 200 g twice daily (8 am and 5 pm) with daily increases of 50 g per feeding until the level of 400 g per feeding (800 g/day) was attained. For the experimental period, starch intake by sheep in three of the groups was decreased abruptly by switching them to different diets. During the experimental period, each group of lambs was assigned randomly to one of four diets (table 1) that had the following N:RFC ratios: (1) .2 (diet A); (2) .4 (diet B); (3) .7 (diet C); and (4) .9 (diet D).

Samples of diets, urine and feces obtained during the experimental period were analyzed. Blood samples were obtained about 3-hr postprandial on the last day of the preliminary period and again 1, 3, 5 and 7 days after the diets were switched for the experimental period. Ruminal fluid was collected about 4-hr postprandial on the last day of the trial.

*Experiment 2.* This experiment was conducted with 18 of the lambs used in experiment 1, and began 22 days after the first experiment ended. Between experiments the lambs were fed 400 g of commercially prepared pellets[4] twice daily. Magnesium balance was determined the last 4 days the lambs were fed the pellets.

For experiment 2, sucrose rather than starch was used to adjust the dietary level of readily fermentable carbohydrate. Table 1 shows the ingredient and chemical composition of the two diets (E and F) formulated for experiment 2. Because we had limited amounts of feed, the trial consisted of a 6-day adjustment period followed by a 4-day collection period.

The lambs were stratified by weight to two groups of nine each. Throughout the 10-day trial, lambs in one group were fed diet E and those in the other group were fed diet F. Diets E and F had N:RFC ratios of about .2 and 1.6, respectively. All lambs were fed 400 g of the appropriate diet twice daily.

Samples of diets, urine and feces collected during the last 4 days of the trial were analyzed. Blood samples were obtained about 3-hr postprandial the last day the lambs were fed pellets and again on days 2, 6 and 10 of the trial. Ruminal fluid was collected about 5-hr postprandial on day 10.

## RESULTS

*Experiment 1.* Ruminal fluid ammonia-nitrogen levels averaged 50, 54, 63 and 67 mg/100 ml for sheep fed diets that had N:RFC ratios of .2, .4, .7 and .9, respectively. Although ruminal ammonia levels increased as starch intake decreased, differences between treatments were not significant ($P>.05$). There was no apparent relationship between ruminal ammonia level and plasma magnesium concentration.

Within treatment groups, plasma magnesium and calcium levels did not differ ($P>.05$) between sampling days. Moreover, plasma magnesium and calcium concentrations were not affected ($P>.05$) by the treatments. Plasma magnesium and calcium levels (mean ± SD) of all sheep averaged 1.8 ± .1 and 10.0 ± .4 mg/100 ml, respectively, during the experimental period.

Plasma potassium concentrations generally increased during the trial, but were not affected ($P>.05$) by decreased intake of starch. Plasma potassium levels (mean ± SD) for lambs in the respective .2, .4, .7 and .9 N:RFC treatment groups were 20.9 ± .7, 21.5 ± .6, 20.9 ± .6 and 21.3 ± .9 mg/100 ml on the last day of the trial.

Magnesium balance data are shown in table 2. Intake, fecal excretion, urinary excretion and retention of magnesium did not differ ($P>.05$) between treatment groups. Values for magnesium absorption were similar for all lambs and averaged only about 1 to 2% of dietary intake.

The treatments had no effect ($P>.05$) on calcium balance. Average values for intake,

[4]Early Market Lamb Pellets, Agway, Inc., Syracuse, NY. Pellets contained per kilogram: magnesium, 5.43 g; calcium, 11.88 g; potassium, 15.43 g; crude protein, 14.9%. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

TABLE 2. MAGNESIUM AND POTASSIUM BALANCE IN LAMBS FED DIETS WITH VARYING RATIOS OF NITROGEN TO READILY FERMENTABLE CARBOHYDRATE (EXPERIMENT 1)

| Diet[a] | N:RFC[b] | Intake | Excretion | | Absorption | Retention |
|---|---|---|---|---|---|---|
| | | | Fecal | Urinary | | |
| | | | Magnesium, mg/day | | | |
| A | .2 | 599 | 592 | 8 | 7 | -1 |
| B | .4 | 606 | 590 | 9 | 16 | 7 |
| C | .7 | 606 | 591 | 13 | 15 | 2 |
| D | .9 | 610 | 593 | 18 | 17 | -1 |
| ± standard error | | 3 | 13 | 4 | 12 | 12 |
| | | | Potassium, g/day | | | |
| A | .2 | 19.29 | 3.15 | 15.12 | 16.14 | 1.02 |
| B | .4 | 19.25 | 3.70 | 15.23 | 15.55 | .32 |
| C | .7 | 19.29 | 3.15 | 16.10 | 16.14 | .04[c] |
| D | .9 | 19.29 | 2.61 | 16.80[c] | 16.68 | -.12[c] |
| ± standard error | | .09 | .44 | .43 | .44 | .29 |

[a]Five wethers per treatment.

[b]Dietary ratio of nitrogen to readily fermentable carbohydrate. All diets contained about 4.1% nitrogen, and diets A, B, C and D contained about 20.5, 10.3, 5.9 and 4.6% readily fermentable carbohydrate, respectively, provided mainly as cornstarch.

[c]Significantly different (P>.05) from diet A value by Dunnett's (Steel and Torrie, 1960) two-sided comparison.

fecal excretion, urinary excretion, apparent absorption and retention of calcium for all sheep were 3.32, 2.58, .08, .73 and .66 g/day, respectively, during the experimental period.

Table 2 shows the effects of decreased intake of readily fermentable carbohydrate on potassium balance. Fecal excretion and apparent absorption of potassium were not affected (P>.05) by the treatment. Urinary potassium excretion was greater (P<.05) in lambs fed diet D than in those fed diet A. Sheep fed diets C and D retained less (P<.05) potassium than those fed diet A.

*Experiment 2.* Ammonia-nitrogen concentration of ruminal fluid was greater (P<.05) in sheep fed the diet without added sucrose (diet F) than in sheep fed supplementary sucrose (diet E). The ammonia levels (mean ± SD) were 30 ± 6 and 42 ± 15 mg/100 ml for sheep fed diets E and F, respectively.

Plasma concentrations of magnesium and calcium are shown in table 3. When switched from pellets to the semipurified diets, magnesium intake decreased markedly, and plasma magnesium concentration declined appreciably; however, magnesium levels were similar for both treatments. Plasma calcium levels tended to decrease during the trial, but were not affected (P>.05) by the treatments. Plasma potassium levels were similar throughout the trial and averaged about 22.0 and 21.6 mg/100 ml for sheep fed diets E and F, respectively.

Table 4 shows the effects of the dietary treatments on magnesium and potassium balance. Fecal excretion of magnesium was lower and urinary excretion, apparent absorption and retention of magnesium were higher in sheep fed diet E than in those fed diet F; however, only apparent absorption (P<.1) and urinary excretion (P<.05) values differed significantly between treatments. Apparent absorption of magnesium averaged about 27% of intake when the lambs were fed pellets, but only about 15 and 2% of intake when they were fed diets E and F, respectively.

Urinary potassium excretion was greater (P<.05) and apparent retention of potassium was lower (P<.05) in sheep fed supplementary sucrose than in those fed the diet without added sucrose (table 4). Fecal excretion and apparent absorption of potassium did not differ significantly between treatments.

Calcium balance data were similar for both treatment groups. Average values for intake, fecal excretion, urinary excretion, apparent absorption and retention of calcium for all sheep were 3.33, 2.85, .15, .48 and .33 g/day, respectively.

TABLE 3. PLASMA MAGNESIUM AND CALCIUM CONCENTRATIONS IN LAMBS FED HIGH-NITROGEN RATIONS WITH (DIET E) AND WITHOUT (DIET F) ADDED SUCROSE (EXPERIMENT 2)[a,b]

| | Plasma concentration, mg/100 ml | | | |
|---|---|---|---|---|
| | Magnesium | | Calcium | |
| Day[c] | Diet E | Diet F | Diet E | Diet F |
| 0 | 2.8 ± .5[d] | 2.6 ± .5[d] | 11.4 ± 1.1 | 11.3 ± .5 |
| 2 | 1.8 ± .2 | 1.6 ± .3 | 10.8 ± 1.4 | 10.4 ± .3 |
| 6 | 1.8 ± .2 | 1.8 ± .3 | 10.4 ± .7 | 10.8 ± .8 |
| 10 | 1.9 ± .2 | 1.8 ± .2 | 10.1 ± .8 | 10.2 ± .5 |

[a]Values are means ± SD (n = 9).

[b]Diets E and F contained about 20.5 and 2.6% readily fermentable carbohydrate, respectively; both diets contained about 4.1% nitrogen.

[c]Day 0 indicates day before lambs were switched from commercially prepared pellets to the semipurified diets. Other days indicate times of sampling during 10-day feeding trial.

[d]Significantly different ($P<.05$) from post-treatment values by Dunnett's (Steel and Torrie, 1960) two-sided comparison.

*Discussion*

Severe hypomagnesemia was not observed in our study, but plasma magnesium levels (1.7 to 1.9 mg/100 ml) were near the lower end of the normal range (1.7 to 3.0 mg/100 ml) reported for sheep (Wilson, 1964). A hypomagnesemic response to intraruminal infusion of ammonium acetate and ammonium carbonate has been observed in cows (Head and Rook, 1955) and lambs (Henry and Cunningham, 1975). However, Moore *et al.* (1972) found that increased intake of nitrogen, provided as either soybean protein alone or with urea, had no effect on blood serum magnesium levels of lambs even though ruminal ammonia levels increased substantially compared to control animals. In addition, other investigators (Christian and Williams, 1960; Wilson, 1963) were unable to induce hypomagnesemia in sheep by markedly increasing their urea intake.

Wilcox and Hoff (1974) suggested that when animals graze forages having a high nitrogen and low readily available carbohydrate content, ruminal ammonia levels may increase rapidly with the consequence of increased ruminal fluid pH and decreased solubility of magnesium. Head and Rook (1955) postulated that high ruminal ammonia levels might interfere with magnesium absorption. In our experiments, ruminal fluid ammonia-nitrogen levels tended to increase as intake of readily fermentable carbohydrate decreased. The ruminal ammonia levels observed in experiment 1 were similar to those reported by Moore *et al.* (1972) for sheep fed high-nitrogen diets. In the study of Moore *et al.* (1972), ruminal ammonia levels increased as nitrogen intake by lambs increased, but apparent absorption of magnesium was not affected. Similarly, in our first experiment with lambs fed high-nitrogen diets, apparent absorption of magnesium was not affected significantly by decreased intake of readily fermentable carbohydrate as starch. However, magnesium absorption was only about 1 to 2% of dietary intake in all treatment groups. With such low values, it is likely that it would be difficult to demonstrate any factor that might markedly lower magnesium absorption.

The reason the apparent availability of magnesium was so low is not known. It could be related to one or more of the dietary ingredients, particularly the relatively high potassium content combined with the low magnesium level. Increased potassium intake has been shown to decrease magnesium absorption, depress urinary magnesium excretion and reduce plasma magnesium concentration (Suttle and Field, 1969; House and Van Campen, 1971; Newton *et al.*, 1972; Fontenot *et al.*, 1973). When the lambs used in our study were fed commercially prepared pellets that contained .5% magnesium and 1.5% potassium, apparent absorption of magnesium was about 27% of dietary intake.

Results on potassium utilization varied between experiments. Potassium retention was least ($P<.05$) in sheep fed the diet with the

TABLE 4. MAGNESIUM AND POTASSIUM BALANCE IN LAMBS FED HIGH-NITROGEN RATIONS WITH (DIET E) AND WITHOUT (DIET F) ADDED SUCROSE (EXPERIMENT 2)[a]

| | | | Excretion | | | |
|---|---|---|---|---|---|---|
| Diet | N:RFC[b] | Intake | Fecal | Urinary | Absorption | Retention |
| | | | Magnesium, mg/day | | | |
| E | .2 | 648 ± 0 | 553 ± 69 | 29 ± 15[c] | 95 ± 69[d] | 66 ± 72 |
| F | 1.6 | 641 ± 15 | 626 ± 111 | 15 ± 12 | 15 ± 111 | -.3 ± 108 |
| | | | Potassium, g/day | | | |
| E | .2 | 19.50 ± 0 | 3.11 ± .78 | 16.32 ± 1.38[c] | 16.39 ± .78 | .07 ± 1.08[c] |
| F | 1.6 | 19.29 ± .42 | 3.43 ± .99 | 14.89 ± 1.35 | 15.86 ± 1.20 | .97 ± .66 |

[a]Values are means ± SD (n = 9).

[b]Dietary ratio of nitrogen to readily fermentable carbohydrate. Both diets contained about 4.1% nitrogen, and diets E and F contained about 20.5 and 2.6% readily fermentable carbohydrate, respectively.

[c]Mean value for diet E significantly different (P<.05) from comparable value for diet F.

[d]Mean value for diet E significantly different (P<.1) from comparable value for diet F.

highest N:RFC ratio in experiment 1, but the opposite was true in experiment 2. In both experiments, low potassium retention was associated with increased ($P<.05$) urinary potassium excretion.

In contrast with experiment 1, our second experiment indicated that magnesium metabolism in sheep may be affected adversely by decreased intake of readily fermentable carbohydrate. Urinary excretion, apparent absorption and retention of magnesium were higher in lambs fed supplementary sucrose than in those fed a diet without added sucrose. Madsen *et al.* (1976) observed similar responses in magnesium utilization in sheep fed supplementary glucose.

Differences in results on magnesium and potassium utilization between experiments reported here could be attributed to the dietary levels and sources of readily fermentable carbohydrate. However, results of the two experiments are not directly comparable because of procedural differences, particularly in the method of adapting the sheep to the experimental rations. Adaptation to urea feeding has been found in lambs (Smith *et al.*, 1960) and is affected by the level of readily fermentable carbohydrate in the diet (McLaren *et al.*, 1965).

Supplementation of ruminant diets with magnesium has become standard practice for preventing hypomagnesemic tetany. Moreover, Wilson *et al.* (1969) suggested that supplementary feeding of starchy grains should reduce the incidence of grass tetany. Although further work is needed on the influence of different carbohydrate supplements on magnesium utilization, results of our second experiment support the view that supplemental energy may enhance the availability of magnesium. The results of our studies do not confirm or refute the hypothesis that an imbalance between crude protein and readily fermentable carbohydrate is an important factor in the etiology of hypomagnesemia.

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